Bone metabolism in rheumatoid arthritis compared with postmenopausal osteoporosis

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SUMMARY Calcium and bone metabolism in 29 rheumatoid arthritis (RA) patients were compared with those in 27 postmenopausal osteoporotic patients. Signs of vitamin D deficiency were found in 20 RA patients, including 12 who took recommended amounts of vitamin D in their diets and were exposed to sufficient sunlight, and in none of the osteoporotic patients. There were no signs of malabsorption. In six out of 15 patients we found increased liver enzyme activity, which may have a role in vitamin D metabolism. We propose the influence of non-steroidal anti-inflammatory drugs (NSAIDs) on vitamin D metabolism in the liver as a possible explanation.

Key words: vitamin D metabolism, vitamin D deficiency, secondary hyperparathyroidism, calcium metabolism, liver enzyme induction, non-steroidal anti-inflammatory drugs.

Osteopenia is a common feature of rheumatoid arthritis (RA). In several studies^{1–5} RA patients have shown decreased bone calcium content and bone density compared with those of matched controls. No difference was found between male and female RA patients.

Several investigators have suggested an underlying disturbance in calcium and vitamin D metabolism as a cause of the osteopenia of RA. Most laboratory evidence for vitamin D deficiency is derived from studies carried out in Great Britain.^{6–8} Vitamin D is a legally obligatory additive to margarine in the Netherlands. A study of vitamin D metabolism among Dutch RA patients was therefore warranted.

This communication reports the results of our study of bone metabolism in RA patients and postmenopausal osteoporotic controls. The interaction between the regulation of calcium metabolism by vitamin D and by parathyroid hormone is studied in more detail.

Patients and methods

PATIENTS

Twenty nine patients, four male and 25 female, with longstanding definite or classical RA were compared with 27 osteoporotic postmenopausal (OP) women. Twenty of the RA patients were seropositive. The diagnostic criteria for OP were radiologic osteopenia, accentuation of the upper and lower margins and vertical trabeculae of the vertebrae (all patients), collapsed vertebrae (18 patients) or other fractures (four patients), or back pain. Other causes of osteopenia were excluded. Patients taking anti-convulsant drugs and those with known liver, kidney, endocrine, or gastrointestinal disorders were excluded from both groups.

METHODS

Serum parameters were obtained after overnight fasting. Analysis was made of serum calcium, inorganic phosphate, total protein, albumin, creatinine, γ -glutamyltranspeptidase activity, alkaline phosphatase activity, vitamin E, folic acid, gastrin, and urinary calcium, creatinine, phosphate, and hydroxyproline. Faeces were collected over a

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72-hour period and weighed. If this weight exceeded 500 g faecal fat content was estimated.

Maximal tubular phosphate reabsorption ($\text{Tm-PO}_4/\text{GFR}$) was calculated by the method of Walton,⁹ and Payne's formula¹⁰ was employed to correct serum calcium concentration for albumin concentration. Values are presented as mean $\pm$ SD unless stated differently.

Serum parathyroid hormone levels (PTH levels) were determined by a radioimmunoassay specific for the C-terminal region. The serum concentration of 25-hydroxyvitamin D (25(OH)D) was measured by a competitive protein binding assay in two different laboratories with nearly identical reference values. The serum level of 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) was determined by a competitive protein binding assay after purification by high-performance liquid chromatography.¹¹ The serum total bile acids after fasting and two hours after loading with chenodeoxycholic acid (1 g) were estimated by an enzymatic spectrophotometric method with 3 α -hydroxysteroid dehydrogenase.^{12, 13} The hydroxylation pattern of the bile acids was determined by a thin-layer chromatographic method.¹⁴ A D-xylose loading test was performed with 25 g D-xylose according to Richterich *et al.*¹⁵ Urinary glucuronic acid concentration was determined before and after loading with D-glucuronolactone (1 g) according to Marsh.¹⁶

X-ray photographs of the pelvis and the thoracic and lumbar spine were performed. Statistical analysis was performed with the two-tailed Student's *t* test ($p < 0.05$). Vertical iliac crest biopsy specimens were obtained after double tetracycline labelling and were processed without decalcification. Biopsy specimens obtained from 22 OP patients were evaluated qualitatively. The biopsy specimens from 17 RA patients were quantitatively analysed by histomorphometric methods.¹⁷ The following parameters were measured in trabecular bone: (a) trabecular bone volume (TBV), i.e., the volume occupied by trabecular bone and osteoid tissue, expressed as a percentage of the total volume between the cortices, (b) osteoid volume (OV), i.e., the volume of osteoid tissue, expressed as a percentage of trabecular bone volume, (c) osteoid surface (OS), i.e., the trabecular surface covered by osteoid seams, expressed as a percentage of total trabecular surface, (d) thickness index of osteoid seams (OV/OS), i.e., an estimation of osteoid thickness calculated as $100 \times \text{OV}/\text{OS}$, (e) resorption surface (RS), i.e., the trabecular surface occupied by resorption lacunae with or without osteoclasts, expressed as a percentage of total trabecular surface, (f) mineralisation rate (M), i.e., the mean distance between double tetracycline labels (each pair measured on

equidistant points) divided by the labelling interval expressed as $\mu\text{m}/\text{day}$.

The results of the histomorphometric measurements were compared with results for controls of similar age and sex from the literature.¹⁸⁻²⁰

Daily protein, calcium, phosphate, and vitamin D intake were recorded by a dietician. Questionnaires were completed for average sunlight exposure and for mobility. The results are given in Table 1.

Results

Disease activity of RA was moderate or severe. The duration ranged from five to 31 years (mean 15.8 years). Eight RA patients were in the Steinbrocker functional capacity grade II, five in II to III, 15 in III, and one in III to IV. Twenty four of the OP patients were in grade II and three in II to III. The ages of the RA patients ranged from 52 to 74 years (mean 64 years), and the ages of the OP patients from 52 to 71 years (mean 69 years). Disease complications in the RA patients were Sjögren's syndrome (14), vasculitis with polyneuropathy (three), Felty's syndrome (two), and cervical myelom compression (two). Eleven had no disease complications.

Eighteen of the 27 OP patients had collapsed vertebrae as compared with five of the 29 RA patients. The x-rays of all OP and 25 RA patients showed osteopenia. Whereas only one OP patient had a low serum calcium level, 23 RA patients had serum calcium levels below 2.25 mmol/l (reference value 2.25-2.60 mmol/l). Disease activity was reflected in low serum albumin levels in 21 of the RA patients. Serum calcium levels were corrected for

Table 1 Comparison of predisposing factors in the osteoporotic (OP) and rheumatoid (RA) groups

Predisposing factor	OP	RA
Mobility (walking)		
0-10 m	—	4
10-100 m	2	25
100 m or more	25	—
Sunlight exposure (h/day)		
0	—	16
0-1	3	8
1 or more	24	5
Vitamin D (IU/day) in diet		
0-100	—	17
100 or more	27	12
Calcium (mg/day) in diet		
0-800	5	12
800 or more	22	17

albumin. There was no difference between RA and OP patients in serum calcium corrected for albumin, in serum creatinine, or in inorganic phosphate.

In 17 RA patients serum alkaline phosphatase activity was increased as shown in Table 2. In contrast with OP patients, in whom 24 h urinary calcium and hydroxyproline excretion were found within the reference range, 22 RA patients had a markedly decreased excretion of calcium (1.9 versus 3.9 mmol/24 h) and increased hydroxyproline excretion (33 versus 19 mg/24 h), suggesting vitamin D deficiency. This was confirmed by serum 25(OH)D levels in the RA patients. These were below normal with a mean value of 23.8 nmol/l. The PTH levels in 14 patients were increased, suggesting secondary hyperparathyroidism. The mean PTH concentration for 27 RA patients was 332 pg/ml; the mean value

obtained in 10 OP patients was 203 pg/ml (see Figs 1 and 2).

Vitamin D intake and sunlight exposure were deficient in 17 RA patients; the remaining 12 patients, whose dietary intake of vitamin D was adequate and who had sufficient sunlight exposure, also had low 25(OH)D levels.

None of the patients showed signs of malabsorption. In 15 RA patients the excretion of glucaric acid was measured before and after oral loading with D-glucuronolactone; in six instances the results indicated liver enzyme induction (Fig. 3).

Bile acid determinations did not show any liver dysfunction. The hydroxylation pattern of the bile acids (not dependent on cytochrome P₄₅₀) did not differ significantly from results obtained in a reference population.

Table 2 Comparison of biochemical values in the osteoporotic (OP) and rheumatoid (RA) groups

Results	Reference value	OP	RA
S* calcium (mmol/l)	2.25–2.60	2.38±0.02	‡2.17±0.02
S. albumin (g/l)	41–45	43.4±0.02	‡37.0±0.7
S. calcium corrected (mmol/l)		2.25±0.03	2.29±0.02
S. alkaline phosphatase (IU/l)	<110	85±4	‡135±7
S. creatinine (µmol/l)	<120	84±2	74±3.5
S. inorganic phosphate (mmol/l)	0.6–1.2	1.21±0.03	1.07±0.03
U.* calcium (mmol/24 h)	4.0–7.0	3.9±0.3	‡1.9±0.3
U. hydroxyproline (mg/24 h)†	20–25	19±1	‡33±2
TmPO ₄ /GFR	0.80–1.35	1.1±0.1	1.03±0.2
S. 25(OH)D (nmol/l)	30	76±6 (n=8) 54±20 (n=3)	23.8±2.7 (n=27)
S. 1,25(OH) ₂ D (pmol/l)	60–160	—	87.1±6.9 (n=9)
S. PTH (pg/ml)	80–270	203±15 (n=9)	332±40 (n=27)
S. vitamin E (µmol/l)	15–35	—	21.5±1.7
S. folic acid (µg/l)	2–7	—	4.33±0.46
S. gastrin (mg/l)	20–130	—	90.1±15.5
U. xylose (g/2 h)	5	—	3.83±0.54
Faeces weight (g/72 h)	500	—	333.7±46.1
S. bile acids fasting (µmol/l)	6.5	—	3.69±0.86
S. bile acids 2 h after loading	14.5	—	11.86±1.76
U. glucaric acid after loading (%)	11–21	—	6.0±2.1

*S=serum; U=urinary.
†SI conversion: mg/24 h × 0.00763=mmol/24 h.
‡Significant by two-tailed Student's *t* test (*p*<0.05).
All values are mean±SEM.
—=not done.

Table 3 Histomorphometric data in patients with rheumatoid arthritis compared with control values from literature^{18–20}

Control values			Rheumatoid arthritis		Significance
	<i>n</i>	Mean±SD	<i>n</i>	Mean±SD	
TBV (%)	27	15.0±3.7	15	13.0±4.4	NS
OV (%)	42	2.0±1.2	17	3.4±2.1	<i>p</i> <0.01
OS (%)	42	10.7±6.3	17	24.3±13.6	<i>p</i> <0.001
OV/OS	42	18.8±4.7	17	14.3±4.7	<i>p</i> <0.01
RS (%)	130	3.6±1.1	17	4.2±2.1	0.05< <i>p</i> <0.10
M (µm/d)	11	0.73±0.22	15	0.66±0.11	NS

Qualitative examination of bone biopsy specimens from the osteoporotic patients showed various degrees of osteopenia, with active remodelling in five and low grade remodelling in 17 samples.

In Table 3 and Fig. 4 the histomorphometric

results in RA patients are compared with control values from literature.¹⁸⁻²⁰

The RA patients had a lower TBV than controls of similar age reported in the literature. However, the size of the group was small and the difference

Fig. 1 Vitamin D metabolites in patients with rheumatoid arthritis (RA).

Fig. 2 Parathyroid hormone levels in osteoporotic (OP) and rheumatoid (RA) patients.

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was not significant. A low TBV was observed in the two patients receiving corticosteroid treatment (8.7 and 8.8% respectively).

The value of the remodelling parameters showed a wide variation. The osteoid volume was increased by more than 5% in three and the osteoid surface

was increased by more than 25% in eight biopsy specimens. However, no biopsy specimen showed an increase in the thickness of osteoid seams.

The mineralisation rate was normal in all biopsy specimens, so that mineralisation disturbance may be excluded. Five biopsy specimens showed evidence of high turnover, i.e., an increased resorption surface and extended osteoid surface.

Discussion

Signs of vitamin D deficiency were found in a large proportion of Dutch patients suffering from longstanding RA, in accordance with British studies.⁶⁻⁸ No vitamin D deficiency was detected in a control group of primary osteoporotic patients. In most cases vitamin D deficiency was accompanied by secondary hyperparathyroidism, as shown by raised parathyroid hormone levels and signs of high turnover in the bone biopsy specimens. Vitamin D deficiency was shown both in RA patients with adequate dietary intake and sufficient sunlight exposure and in those for whom indications of insufficient dietary intake and low sunlight exposure were obtained. In none of the patients was malabsorption found. A disturbance of vitamin D metabolism may therefore be involved in RA. In pathological circumstances 1,25(OH)₂D levels are maintained in the normal range as long as possible. There are no tests available for direct investigation of vitamin D metabolism in the liver. However, we did find indications of liver enzyme induction in six out of a total of 15 patients by an indirect test. Low 25(OH)D levels may be due to the influence of NSAIDs on vitamin D metabolism in the liver. It is possible that NSAIDs induce an increased enzymatic conversion of active vitamin D metabolites in the liver, as do anticonvulsant drugs.²¹ We are performing antipyrine loading tests in these patients in order to investigate further liver metabolic rate (hydroxylation activity dependent on cytochrome P₄₅₀).

Histomorphometry of the bone biopsy specimens showed osteopenia both in the RA patients and in the primary osteoporotic patients.²² In 30% of the RA patients there was evidence of a high turnover. Increased bone turnover was also observed in RA patients with technetium bone scintigraphy.²³⁻²⁵ The high turnover is probably a manifestation of secondary hyperparathyroidism shown by the raised parathyroid hormone levels. Overt osteomalacia was excluded by the normal osteoid thickness index and the normal mineralisation rate in the bone biopsy specimens.¹⁷ It may be assumed that vitamin D deficiency, by decreasing calcium absorption and serum calcium levels, stimulates parathyroid hormone production to restore calcium levels. The

Fig. 3 Glucaric acid excretion after oral loading with 1 g D-glucuronolactone (expressed in % conversion) in 15 rheumatoid (RA) patients.

secondary hyperparathyroidism stimulates renal calcium reabsorption, thus minimising urinary calcium loss.

The histological picture of high turnover agrees with that observed by others in incipient vitamin D deficiency.²⁶ Only in later stages will true osteomalacia be found. The hyperparathyroidism may increase bone loss.¹⁷ Our results contrast with the

findings in the recent study of Ng *et al.*²⁷ These investigators observed no signs of osteomalacia or secondary hyperparathyroidism in RA bone biopsy specimens. The 25(OH)D levels were much higher in their patients than in ours. Only 9% of their patients had negligible sunlight exposure as opposed to 55% of the patients in our study. This difference is probably due to the fact that their study included

Fig. 4 Histomorphometric data in rheumatoid (RA) patients compared with controls of similar age from the literature.¹⁸⁻²⁰

younger patients, whereas our patients were all over 50 years of age.

RA patients have a greater bone loss than osteoporotic patients, especially those taking corticosteroids.²⁷ RA patients taking corticosteroids also have a greater bone loss than patients with bronchial asthma on corticosteroids, the bone loss occurring earlier in the course of the disease.¹⁹ Only six of the RA patients investigated had been taking or were on corticosteroids. No difference was found between them and the other RA patients with regards to calcium metabolism.

In conclusion our results indicate that in long-standing RA vitamin D deficiency accompanied by secondary hyperparathyroidism is an additional factor contributing to the severity of osteopenia. Circumstantial evidence suggests that disturbed turnover of 25(OH)D in the liver may have a role.

Questions remaining to be answered are whether this disturbance of calcium metabolism is dependent on the severity of the rheumatoid process and whether there is a contributing influence of NSAIDs on metabolism of vitamin D in the liver.

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